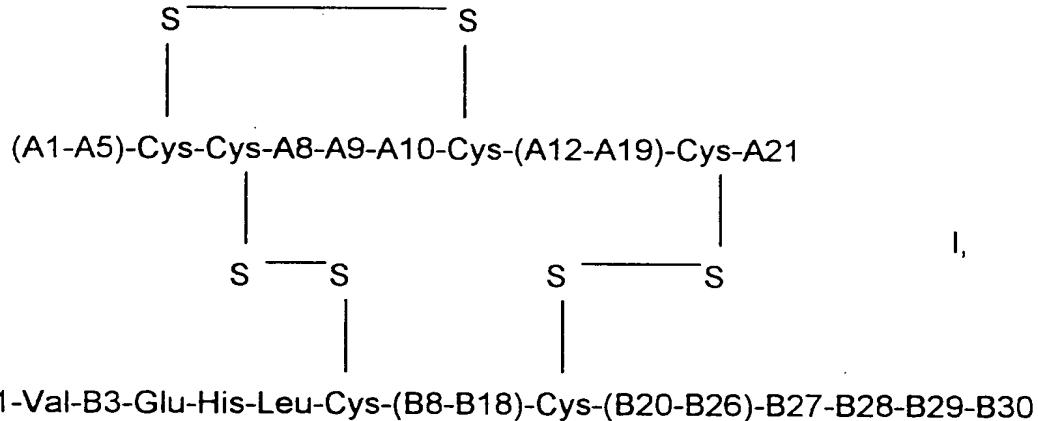


We claim
1 Patent claims

1. An insulin derivative or a physiologically tolerable salt thereof, in which asparagine (Asn) in position B3 of the B chain is replaced by a naturally occurring basic amino acid residue and at least one amino acid residue in the positions B27, B28 or B29 of the B chain is replaced by another naturally occurring neutral or acidic amino acid residue, it optionally being possible for asparagine (Asn) in position 21 of the A chain to be replaced by Asp, Gly, Ser, Thr or Ala and for phenylalanine (Phe) in position B1 of the B chain and the amino acid residue in position B30 of the B chain to be absent.

2. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 1, of formula I

15



25 in which

(A1-A5) are the amino acid residues in the positions A1 to A5 of the A chain of human insulin or animal insulin,

30 (A12-A19) are the amino acid residues in the positions A12 to A19 of the A chain of human insulin or animal insulin,
 A21 is Asn, Asp, Gly, Ser, Thr or Ala,

33

(B8-B18) are the amino acid residues in the positions B8 to B18 of the B chain of human insulin or animal insulin,

(B20-B26) are the amino acid residues in the positions B20 to B26 of the B chain of human insulin or animal insulin,

5 A8, A9, A10 are the amino acid residues in the positions A8, A9 and A10 of the A chain of human insulin or animal insulin,

10 B30 is -OH or the amino acid residue in position B30 of the B chain of human insulin or animal insulin,

B1 is a phenylalanine residue (Phe) or a hydrogen atom,

15 B3 is a naturally occurring basic amino acid residue,

B27, B28

and B29 are the amino acid residues in the positions B27, B28 and B29 of the B chain of human insulin or animal insulin or in each case are another naturally occurring amino acid residue, where at least one of the amino acid residues in the positions B27, B28 and B29 of the B chain is replaced by another naturally occurring amino acid residue which is selected from the group consisting of the neutral or acidic amino acids.

20

25

3. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 2, wherein

A8 is alanine (Ala),

30 A9 is serine (Ser),

A10 is valine (Val) and

B30 is alanine (Ala).

34

4. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 2, wherein
A8 is threonine (Thr),
5 A9 is serine (Ser) and
A10 is isoleucine (Ile).

5. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 4, wherein
10 B30 is alanine (Ala).

6. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 4, wherein
B30 is threonine (Thr).
15

7. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 6, wherein
(A1-A5) are the amino acid residues in the positions A1 to A5 of the A chain of human insulin,
20

(A12-A19) are the amino acid residues in the positions A12 to A19 of the A chain of human insulin,

25 (B8-B18) are the amino acid residues in the positions B8 to B18 of the B chain of human insulin and

(B20-B26) are the amino acid residues in the positions B20 to B26 of the B chain of human insulin.
30 8. An insulin derivative or a physiologically tolerable salt thereof as claimed in one or more of claims 1 to 7, wherein the amino acid residue in position B1 of the B chain is a phenylalanine residue (Phe).

9. An insulin derivative or a physiologically tolerable salt thereof as claimed in ⁽¹⁹⁾~~one or more of claims 1 to 8~~, wherein the amino acid residue in position B3 of the B chain is a histidine (His), lysine (Lys) or arginine residue (Arg).

10

10. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 9, wherein the amino acid residue in position B3 of the B chain is a histidine residue (His).

11. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 9, wherein the amino acid residue in position B3 of the B chain is an arginine residue (Arg).

15

12. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 9, wherein the amino acid residue in position B3 of the B chain is a lysine residue (Lys).

13. An insulin derivative or a physiologically tolerable salt thereof as claimed in ⁽¹⁹⁾~~one or more of claims 1 to 12~~, wherein at least one of the amino acid residues in the positions B27, B28 and B29 of the B chain is a naturally occurring amino acid residue which is selected from the group consisting of isoleucine (Ile), aspartic acid (Asp) and glutamic acid (Glu).

20

25

14. An insulin derivative or a physiologically tolerable salt thereof as claimed in ⁽¹⁹⁾~~one or more of claims 1 to 13~~, wherein at least one of the amino acid residues in the positions B27, B28 and B29 of the B chain is a naturally occurring amino acid residue which is selected from the group consisting of the acidic amino acids.

30

15. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 14, wherein at least one of the amino acid residues in the

positions B27, B28 and B29 of the B chain is an aspartic acid residue (Asp).

16. ¹⁹ An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 14, wherein at least one of the amino acid residues in the positions B27, B28 and B29 of the B chain is a glutamic acid residue (Glu).

17. ²⁵ An insulin derivative or a physiologically tolerable salt thereof as claimed in ^{Claim 19} ~~one or more of claims 1 to 13~~, wherein at least one of the amino acid residues in the positions ^{B27 and B28} of the B chain is replaced by a naturally occurring amino acid residue which is selected from the group consisting of the neutral amino acids.

18. ²⁶ An insulin derivative or a physiologically tolerable salt thereof as claimed in claim ²⁵ 17, wherein at least one of the amino acid residues in the positions B27, B28 and B29 of the B chain is an isoleucine residue (Ile).

19. ¹⁶ An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 15, wherein the amino acid residue in position B27 of the B chain is an aspartic acid residue (Asp).

20. ¹⁷ An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 15, wherein the amino acid residue in position B28 of the B chain is an aspartic acid residue (Asp).

21. ¹⁸ An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 15, wherein the amino acid residue in position B29 of the B chain is an aspartic acid residue (Asp).

22. ²⁰ An insulin derivative or a physiologically tolerable salt thereof as claimed in claim ¹⁹ 16, wherein the amino acid residue in position B27 of the B chain is a glutamic acid residue (Glu).

21

28. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 19, wherein the amino acid residue in position B28 of the B chain is a glutamic acid residue (Glu).

5 22

24. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 19, wherein the amino acid residue in position B29 of the B chain is a glutamic acid residue (Glu).

10 27

25. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 26, wherein the amino acid residue in position B28 of the B chain is an isoleucine residue (Ile).

15 33

26. An insulin derivative or a physiologically tolerable salt thereof as claimed in ^{CLAIM} ~~one or more of claims 1 to 25~~, wherein the amino acid residue in position A21 of the A chain is an asparagine residue (Asp).

20 34

27. An insulip derivative or a physiologically tolerable salt thereof as claimed in claim 26, wherein the A chain has the sequence

Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asp (SEQ ID NO.: 9)

25

and the B chain has the sequence

Phe Val Lys Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Ile Lys Thr (SEQ ID NO.: 10).

30 23

28. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim 24, wherein the B chain has the sequence

Phe Val Lys Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu

Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Glu Thr

(SEQ ID NO 3).

5 ~~30~~ 31. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim ~~16~~²⁶, wherein the amino acid residue in position B27 of the B chain is an isoleucine residue (Ile).

10 ~~31~~ 30. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim ~~30~~³⁰, wherein the B chain has the sequence

Phe Val Lys Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu
Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Ile Pro Lys Thr

15 (SEQ ID NO 5).

~~28~~ 31. An insulin derivative or a physiologically tolerable salt thereof as claimed in claim ~~26~~²⁷, wherein the B chain has the sequence

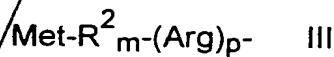
20 Phe Val Lys Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu
Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Ile Lys Thr

(SEQ ID NO 4).

25 32. A process for the preparation of an insulin derivative or of a physiologically tolerable salt thereof as claimed in ~~one or more of claims 1 to 30~~^{Claim 1}, comprising the construction of a replicable expression vehicle which contains a DNA sequence which codes for a precursor of the insulin derivative, in which the amino acid residue in position A1 of the A chain is linked to the amino acid residue B30 of the B chain via a peptide chain of the formula II



in which R^1_n is a peptide chain having n amino acid residues and n is an integer from 0 to 34, and the B chain is prolonged in position $B1$ by a 5 peptide chain of the formula III



in which R^2_m is a peptide chain having m amino acid residues, m is an 10 integer from 0 to 40 and p is 0, 1 or 2, expression in a host cell and release of the insulin derivative from its precursor using chemical and/or enzymatic methods.

15 ~~38.~~ ⁴⁶ The process as claimed in claim ~~32~~, wherein the host cell is a bacterium.

~~39.~~ ⁴⁷ The process as claimed in claim ~~33~~, wherein the bacterium is ⁴⁶ *E. coli*.

20 ~~36.~~ ⁴⁸ The process as claimed in claim ~~32~~, wherein the host cell is a yeast.

~~39.~~ ⁴⁹ The process as claimed in claim ~~35~~, ⁴⁵ wherein the yeast is ⁴⁸ *Saccharomyces cerevisiae*.

25 37. The process as claimed in one of claims 32 to 36 for the preparation of an insulin derivative as claimed in claim 26, wherein the precursor of the insulin derivative has the sequence

Met Ala Thr Thr Ser Thr Gly Asn Ser Ala Arg Phe Val Lys Gln
 30 His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys
 Gly Glu Arg Gly Phe Phe Tyr Thr Ile Lys Thr Arg Arg Glu Ala

Glu Asp Pro Gln Val Gly Gln Val Glu Leu Gly Gly Pro Gly
Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys
Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr
Gln Leu Glu Asn Tyr Cys Asp

5

(SEQ ID NO.: 11).

38. The process as claimed in one of claims 32 to 36 for the preparation
of an insulin derivative as claimed in claim 28, wherein the precursor of the
10 insulin derivative has the sequence

Met Ala Thr Thr Ser Thr Gly Asn Ser Ala Arg
Phe Val Lys Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu
Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Glu Thr
15 Arg Arg Glu Ala Glu Asp Pro Gln Val Gly Gln Val Glu Leu Gly
Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly
Ser Leu Gln Lys Arg
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln
Leu Glu Asn Tyr Cys Asn

20

(SEQ ID NO 6).

39. The process as claimed in one of claims 32 to 36 for the preparation
of an insulin derivative as claimed in claim 30, wherein the precursor of the
25 insulin derivative has the sequence

Met Ala Thr Thr Ser Thr Gly Asn Ser Ala Arg
Phe Val Lys Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu
Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Ile Pro Lys Thr

Arg Arg Glu Ala Glu Asp Pro Gln Val Gly Gln Val Glu Leu Gly
Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly
Ser Leu Gln Lys Arg
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln
5 Leu Glu Asn Tyr Cys Asn

(SEQ ID NO 8).

40. The process as claimed in one of claims 32 to 36 for the preparation
10 of an insulin derivative as claimed in claim 31, wherein the precursor of the
insulin derivative has the sequence

Met Ala Thr Thr Ser Thr Gly Asn Ser Ala Arg
Phe Val Lys Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu
15 Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Ile Lys Thr
Arg Arg Glu Ala Glu Asp Pro Gln Val Gly Gln Val Glu Leu Gly
Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly
Ser Leu Gln Lys Arg
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln
20 Leu Glu Asn Tyr Cys Asn

(SEQ ID NO 7).

41. A precursor of the insulin derivative as claimed in claim 32.

25 42. A precursor of the insulin derivative as claimed in claim 36.

43. A precursor of the insulin derivative as claimed in claim 39.

44. A precursor of the insulin derivative as claimed in claim 40.

45. A DNA sequence which codes for a precursor of the insulin derivative as claimed in claim 41.

5 46. A DNA sequence which codes for a precursor of the insulin derivative as claimed in claim 42.

10 47. A DNA sequence which codes for a precursor of the insulin derivative as claimed in claim 43.

15 48. A DNA sequence which codes for a precursor of the insulin derivative as claimed in claim 44.

20 49. An expression vehicle comprising a DNA sequence as claimed in claim 45.

50. An expression vehicle comprising a DNA sequence as claimed in claim 46.

25 51. An expression vehicle comprising a DNA sequence as claimed in claim 47.

52. An expression vehicle comprising a DNA sequence as claimed in claim 48.

58 53. A host cell which is transformed using an expression vehicle as claimed in ^{Claim 49 to 52} ~~one of claims 49 to 52~~.

36 54. A pharmaceutical preparation, which comprises at least one insulin derivative and/or a physiologically tolerable salt thereof as claimed in ^{Claim 1} ~~one or more of claims 1 to 30~~.

37 56. A pharmaceutical preparation as claimed in claim 54, which comprises the insulin derivative and/or the physiologically tolerable salt thereof in dissolved, amorphous and/or crystalline form.

38 5. 56. A pharmaceutical preparation as claimed in claim 54, which further comprises a depot auxiliary.

39 10 57. A pharmaceutical preparation as claimed in claim 58, wherein the depot auxiliary is protamine sulfate, where the insulin derivative and/or the physiologically tolerable salt thereof is present with the protamine sulfate in a cocrystallizate.

41 15 58. An injectable solution having insulin activity, comprising the pharmaceutical preparation as claimed in ~~one of claims 54 to 56~~ in dissolved form.

59. The use of the insulin derivative and/or its physiologically tolerable salt as claimed in one or more of claims 1 to 31 for the production of a pharmaceutical preparation which has an insulin activity with a rapid onset of action.

Add A47
Add D57